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Original Article

Vitamin E plays a protective role while acrylamide administration disrupted the placenta structure in pregnancy: An experimental study

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Abstract

Aim: The present study aimed to investigate possible variations in the placental tissues of rats upon acrylamide (AA) and vitamin E (Vit E) applications between the 0th and 20th days of pregnancy.

Materials and Methods: Pregnant rats were divided into control, corn oil, AA, Vit E, AA + Vit E groups. At the end of the experimental period, the abdominal tissue of pregnant rats that were anesthetized with xylazine-ketamine was incised, and the placental tissues that connect the mother and the fetus were removed. Biochemical analyses were conducted based on the malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), protein, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) levels in placental tissues.

Results: It was observed that there were no differences between the control and corn oil groups. It was determined that AA administration increased MDA, TOS and OSI levels and decreased SOD, CAT, TAS, and GSH levels when compared to all other groups. Vit E administration increased GSH, TAS, SOD and CAT levels when compared to all other groups. In the AA + Vit E group, it was observed oxidative stress parameters approached control group levels.

Conclusion: Administration of 10 mg/kg/bw AA led to placental oxidative stress in pregnant rats. This action induced by AA was not only through the increase in MDA, TOS and OSI levels, but also through the reduction of GSH, SOD, CAT, and TAS levels, leading to the deterioration of the oxidant-antioxidant balance. Administration of 100 mg/kg/bw Vit E increased antioxidant capacity in placental tissue. To minimize AA-induced toxicity exposure, we recommend the consumption of sufficient Vit E throughout life not only during pregnancy.

Keywords: Placenta; acrylamide; vitamin E; pregnancy; oxidative stress; rat

INTRODUCTION

Acrylamide (AA) is a water-soluble synthetic chemical substance. AA could be found in almost all industries. It is widely used in biochemistry, biotechnology, molecular biology laboratories (i.e., in electrophoresis, chromatography). It is also used during production in several industries such as textile, cosmetics, and hygiene (1). Furthermore, AA forms in food that contains monosaccharide and asparagine amino acid. Although the said formation mechanism was not described, acrylamide is produced as a result of mallard reaction spontaneously at 120oC and above temperatures. AA is produced in foods especially during frying, grilling, or baking. Due to this mechanism, AA is studied extensively (2,3). The studies reported that AA had n neurotoxic, carcinogenic, genotoxic, developmental retardation effects and effects on the reproductive system of experimental animals (4-7). Indigested AA could reach all tissues since it is easily soluble in water. This leads to the disruption of the oxidant / antioxidant balance in the system. Oxidants such as AA trigger oxidative stress through the increase in lipid peroxidation and reduction in glutathione. Oxidative stress could also lead to major cellular and tissue damages (8-12).

Vitamin E belongs to the group of fat-soluble vitamins. It could be found in 4 different forms and the most active form is α (alpha) tocopherol for humans. Wheat extract, sunflower seed, and oil, corn and soybean oil, olive oil, green olives, nuts, peanuts, and almonds are rich in vitamin E. Vitamin E exhibits a very strong antioxidant effect. It converts free radicals such as H2O2, O2, OH- into less reactive compounds. Thus, it prevents oxidative

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stress-induced cellular damage (13-15).

The present study aimed to investigate the effects of AA and Vitamin E administration on placental tissues in pregnant rats.

MATERIALS and METHODS

Ethical approval was obtained from the İnönü University, Faculty of Medicine Experimental Animals Ethics Committee (2016 / A-24). Young female Wistar albino rats that weighed 250 ± 20 g bred at İnönü University, Faculty of Medicine Experimental Animal Breeding and Research Center (INÜTF-DEHÜM) were used in the study. The rats were placed in special cages at 5 pm in the rate of a male rat to every 2 females. They were kept in the same cage until 8 am the next day. At the end of this period, the males were separated from the females. Vaginal smears were taken from female rats and examined under a microscope and females with sperm on the smear were accepted as half-day pregnant. Females whose pregnancies were not + in the smear test were excluded from the experiment. Pregnant rats were kept in INÜTF-DEHÜM rooms under 21 ± 2ºC for 20 days (gestation period), under 12 hours of daylight and 12 hours of darkness and the rooms were constantly ventilated by aspirators. The rats were fed ad-libitum during the experiment. Forty rats determined as pregnant with the smear test were randomly selected and divided into 5 groups as follows:

The Study Design

Group 1: Control group (n = 8), no administration was conducted to pregnant rats mated simultaneously with the experimental group rats.

Group 2: Corn oil group (n = 8), corn oil was administered to pregnant rats via oral gavage.

Group 3: AA group (n = 8), 10 mg/kg/BW AA was solved in drinking water and administered to pregnant rats via oral gavage at (Sigma A8887) (16).

Group 4: Vit E group (n = 8), 100 mg/kg/BW Vit E was dissolved in corn oil and administered to pregnant rats via oral gavage (Sigma T3251) (17).

Group 5: AA + Vit E group (n = 8), 10 mg/kg/bw AA and 100 mg/kg/bw Vit E were administered to pregnant rats via oral gavage.

The applications were all 1 mL and administered at the same hour on all days during the 20-day experimental period. Placental tissues were removed by cesarean section under anesthesia on the 20th day of gestation.

Preparation of the Tissues for Biochemical Analyses

Placental tissues preserved in the freezer (-80°C) were removed on the day of the analysis and weighed. Phosphate buffer was added to create a 10% homogenate and homogenized on ice for 1-2 minutes at 12000 rpm (IKA, Germany). The homogenate products were used to determine the malondialdehyde (MDA) levels. Serum samples were obtained by centrifuging the tissue homogenates at 5000 rpm and +4 degrees for 30 minutes. The serum samples were used to determine the reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), total antioxidant status (TAS), total oxidant status (TOS) oxidative stress index (OSI) and protein levels and various biochemical parameters.

MDA Levels

MDA analysis was conducted with the method developed by Uchiyama and Mihara (18). The MDA concentration was determined by the measurement of the supernatant extracted from the n-butanol phase of the pink-colored product formed by the reaction between the MDA in the supernatant and thiobarbituric acid at 535 and 520 nm with a spectrophotometer. Results were expressed as nmol/g wet tissue.

GSH Levels

GSH analysis was conducted with the method described by Ellman (19). The GSH concentration in the analysis tube produces a yellow-green color reacting with 5,5-dithiobis 2-nitrobenzoic acid, and GSH concentration is determined by reading the light intensity of this color in a spectrophotometer at 410 nm wavelength. Results were given as nmol/g wet tissue.

SOD Activity

The method developed by Sun et al. (20) was used to determine SOD enzyme activity. In the method, superoxide radicals are produced by the xanthine-xanthine oxidase, leading to the formation of a blue color induced by NBT (nitro blue tetrazolium) reduction. The SOD activity is determined by the absorbance of the formazan at 560 nm. Results are presented as U/g protein

CAT Activity

Aebi and Bergmeyer's (21) method was used to determine the CAT enzyme activity. The maximum absorbance initiated by hydrogen peroxide (H2O2) in the ultraviolet spectrum is observed at 240 nm and the hydrogen peroxidase, added to the medium, breaks down into water and oxygen by catalase, leading to a reduction in absorbance at 240 nm. The enzyme activity is determined by the measurement of the reduction in absorbance for 1 minute. The findings are expressed as K/g protein.

Protein Levels

The method developed by Lowry et al. (22) was used to determine the pancreas tissue serum sample total protein content at 700 nm. Results are given as mg/mL.

TOS Levels

TOS was determined by the method developed by Erel (23). Based on this method, absorbance of 500 µL reagent 1 (measurement buffer) and 75 µL serum mixture was determined at 530 nm with ELISA set to 25oC. In the next stage, the product was incubated for 10 min after the addition of 25 µL reagent 2 (pro-chromogenic solution). Then, the absorbance was read again at 530 nm to

determine the TOS (27). Results are given as micromole H2O2 Equiv./l.

TAS Levels

TAS was determined by the method developed by Erel (24). Based on this method, absorbance of 500 µL reagent 1 (measurement buffer) and 30 µL serum mixture was determined at 660 nm with ELISA set to 25oC. In the next stage, the product was incubated for 10 min after the addition of 75 µL reagent 2 (colored ABTS solution). Then, the absorbance was read again at 660 nm to determine the TAS (28). Results are given as millimole Trolox Equiv./l.

OSI Levels

OSI is the rate of TOS to TAS (23). It was calculated with the following formula: OSI (arbitrary unit) = TOS (micromole H2O2 Eqv/L) / TAS (millimole Trolox Eqv/L) X 10.

Statistical Analysis

Statistical analyses were conducted with SPSS 21.0 for Windows software. Data were presented using mean and standard deviation values. Lavine test was used to determine the homogeneity of variances between the groups. One-way ANOVA and Tukey HSD post-hoc analysis were used for homogenous group variances, while Welch test and Tamhane's T2 post-hoc analysis were used for non-homogenous group variances. The confidence interval was determined as 0.05.

RESULTS

We did not determine any difference between the control and corn oil groups. We found that AA administration during pregnancy increased MDA, TOS, OSI levels and decreased GSH, TAS, SOD and CAT levels in placental tissue when compared to all other groups. In the Vit E group, GSH, SOD, CAT and TAS levels increased when compared to all other groups. It was observed that MDA, TOS, OSI levels significantly decreased, and TAS, GSH, SOD, CAT levels increased with AA + Vit E administration when compared to AA group (P <0.001) (Tables 1, 2).

Table 1. Placenta tissue oxidant–antioxidant parameters in all groups.

Groups	MDA (nmol/gwt)	GSH (nmol/gwt)	SOD (U/g protein)	CAT (K/g Protein)
C	378.5±10.57^{a}	732.06±23.78^{a}	34.7±2.61^{a}	4.04±0.08^{a}
Co	364.72±7.59^{a}	744.76±26.24^{a}	37.48±1.77^{a}	4.06±0.17^{a}
AA	479.23±13.77^{b}	524.58±13.61^{b}	24.14±2.7^{b}	2.71±0.14^{b}
Vit E	322.33±5.79^{c}	944.57±33.92^{c}	60.63±1.71^{c}	6.49±0.3^{c}
AA+ Vit E	405.33±2.79^{d}	839.01±26.48^{d}	44.7±1.25^{d}	5.4±0.18^{d}
p	<0.001	<0.001	<0.001	<0.001

Data are expressed as mean and standard deviation. MDA; Malondialdehyde, GSH; reduced glutathione, SOD; superoxide dismutase, CAT; catalase, gwt; gram wet tissue. Groups: Control (C), Corn Oil (CO), Acrylamide (AA), E Vit (Vitamine E), AA+ Vit E (Acrylamide + Vitamine E). (n = 10). The groups with different superscripts represent the statistical significance (p < 0.001).

Table 2. Pancreas Tissue Oxidative stress index parameters of all groups

Groups	TAS (mmol/L)	TOS (µmol/L)	OSI (AU)
C	1.22±0.07^{a}	28.25±1.48^{a}	239±23.25^{a}
CO	1.19±0.06^{a}	27.75±1.8^{a}	247±21.99^{a}
AA	0.56±0.06^{b}	42.88±2.35^{b}	787.38±111.26^{b}
E Vit	1.83±0.06^{c}	23.89±1.36^{c}	135.5±8.09^{c}
AA +E Vit	1.49±0.09^{d}	34.66±1.28^{d}	240.75±11.79^{a}
p	<0.001	<0.001	<0.001

Groups: Control (C), Corn Oil (CO), Acrylamide (AA), E Vit (Vitamine E), AA+ Vit E (Acrylamide + Vitamine E). (n = 10). The groups with different superscripts represent the statistical significance (p < 0.001).

DISCUSSION

Since AA is water-soluble, it can penetrate rat and mouse placenta. It reaches fetal tissues and could lead to permanent damages based on daily consumption (8). Since AA was categorized as a 2A carcinogen substance for humans recently, the number of experimental studies on AA in both adults and fetuses has been increased. Certain studies reported that acrylamide administration leads to serious biochemical, histopathological, genetic and morphological abnormalities on various fetal tissues based on the dose and duration (9, 10, 25, 26).

In previous studies, 10 mg/kg AA was administered to the rats between the 7th and 28th days of pregnancy and its effects on brain tissues were investigated. After 28 days, an increase in MDA levels, which is an oxidative stress parameter, and a decrease in GSH levels was observed in the fetal rat brain tissues when compared to all other

groups (10). In another pregnancy study, between the 6th and 19th days of pregnancy, different (50, 100, 200 ppm) of AA were administered to the drinking water of pregnant rats. It was reported that the administration of 200 ppm AA increased MDA levels, which is the final marker of lipid peroxidation, in fetal brain tissues (27). Erdemli et al. (26) reported that 5mg/ kg /day AA administration during pregnancy statistically significantly increased MDA and TOS levels in brain tissues when compared to all other groups and 100mg/kg/day Vit E administration returned stress parameters to the control group levels. Studies on brain tissues during pregnancy reported that AA administration shifted the oxidant/antioxidant balance in favor of oxidants and led to oxidative stress. During pregnancy, oral AA administration reached the fetal brain tissues through the placenta, penetrated the blood-brain barrier, leading to oxidative stress-induced damages. Erdemli et al. investigated the maternal and fetal rat kidney tissues in a study where 10 mg/kg/BW AA and 100 mg/kg/BW vitamin E were administered in the pregnancy model. In the AA administrated group, MDA and TOS levels increased in both maternal and fetal kidney tissues when compared to the other groups, while Vit E administration increased GSH, TAS, SOD, and CAT levels when compared to all other groups (6). Yu et al. administered 10 and 50 mg/kg/day AA to rats between the 3rd and 13th days of pregnancy. On the 13th day of pregnancy, the rat placental tissues were removed and examined. The authors reported that, depending on the dose, AA reduced mRNA levels such as Esx1, Hand1, and Hand2 that play key roles in the placental tissue and lead to histopathological problems (28).

In a study that investigated whether Vit E had a protective role, the authors administered 100 mg/kg Vit E to diabetic male rats for 6 weeks. The rat brain tissues were examined after 6 weeks. The authors reported that hippocampus tissue TAS levels increased when compared to the diabetes group and TOS levels decreased with Vit E administration (29). Erdemli et al. (30) administered 5 mg/ kg/BW AA and 100 mg/kg/BW Vit E daily as a preservative throughout pregnancy. On the 20th day of pregnancy, rat placental tissues were removed with the cesarean section under anesthesia. Biochemical analysis of the placental tissue demonstrated that AA administration increased MDA, XO and TOS levels when compared to all other groups. It was found that Vit E administration increased TAS and GSH levels when compared to all other groups. They reported that AA increased the oxidant capacity in placental tissues, while Vit E improved the antioxidant capacity and inhibited oxidative stress. The present study findings were consistent with other reports.

CONCLUSION

In our previous pregnancy model, we reported that 5 mg/ kg/bw AA dose administration led to oxidative stress in the placental tissue shifted the oxidant/antioxidant balance in favor of oxidants. In the present study, the investigation of the effects of 10 mg/kg/bw AA dose on placental tissues demonstrated that the toxic material filtration potential of the maternal liver and kidney tissues were insufficient based on more detailed biochemical parameters. Thus, we recommend that strong antioxidant substances such as Vit E should be consumed daily to protect the mother and the fetus from permanent damages induced by food-borne AA toxicity.

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